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Preventing or treating epithelial tissue damage or hair loss

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Preventing or treating epithelial tissue damage or hair loss

The present invention pertains to a method for preventing and/or treating epithelial tissue damage, such as is effected by inflammatory reactions, ageing or cancer and/or to prevent and/or treat hair loss. In particular, the present invention relates to substances and/or compositions modifying, in particular reducing/inhibiting/blocking endogenous CD_{1d} function. According to another aspect the present invention also provides a method for screening for compounds suitable for use in the method and the composition of the present invention.

The most prominent epithelial tissue in living beings is the skin, which represents the largest organ in the organism. The system of skin integument, which comprises the epidermis, dermis and the stratum cornium, correlates with those of internal organs and concurrently interacts with the surroundings. Being the interface between the environment and organism itself, the skin is heavily influenced by external factors and also variable parameters of the organism's inner system. The skin's regulative mechanisms need, therefore, always be active to induce systemic changes necessary to maintain normal pathological events concerning skin integument morphology and activities. A great deal of processes assuring the adequate consumption of increased affluence of energetic and plastic substances according to the skin's needs become guarantors of morphological and functional stability of skin structures. So, the state of integuments determines the realization of metabolic processes necessary for skin cell viability and activity leading to the presence of healthy skin peculiarities such as barrier function, elasticity, turgor properties, humidity, pigmentation etc..

During the lifetime of a living being different signs, characteristic of ageing, appear on the skin, with the principal clinical signs being the appearance of fine lines and deep wrinkles which increase or are accentuated with age. Moreover, the skin's complexion is generally modified and diffuse irritations and occasionally telangiectasias may come into existence on

certain areas.

These signs of ageing are even promoted by exposure of the skin to exogenous influences, such as e.g. UV-radiation, pollutants, free radicals or chemical substances.

Moderate UV exposure generally causes the well known effects of reddening the skin with an accompanying inflammation reaction, known as erythema. This phenomenon, often referred to as "sunburn", is painful and commonly results in a subsequent peeling of the skin.

Moreover, excessive UV-exposure of the skin may also lead to the onset of severe disorders, such as carcinogenesis, the most common tumours being the basal cell carcinoma (BCC), followed by squamous cell carcinoma (SCC), and more rarely malignant melanoma. Apart from damages on the DNA-level also immuno-suppression caused by UV exposure seems to account for both, non-melanoma and melanoma cancer promotion. It is presently acknowledged that photo-induced immuno-suppression permits the initiated tumour cell to evade recognition and rejection by normal immunological mechanisms, to remain latent for extended periods, and to eventually proliferate into a tumour. This concept concurs with the findings that immuno-compromised patients, whether genetically (xeroderma pigmentosum) or pharmacologically, such as e.g. organ transplant recipients, have a higher incidence of skin cancer as compared to people with a properly functioning immune system.

In the art several means have been proposed to prevent destructive effects of environmental factors on epithelial cells, in particular skin epithelial cells.

As regards protection to sun radiation "sun blocks" or "sunscreens" have been made available, which are applied to the skin prior to sun exposure. Typically, sunscreen compositions contain chemical agents, such as certain benzophenones, dibenzylmethanes or substituted para-aminobenzoates, i.e. compounds absorbing ultraviolet radiation, so that it cannot penetrate the skin. However, some of the compounds used for this purpose have shown to lack sufficient light stability and may even become toxic over long term application. In addition, they must stay continuously on the surface of the skin at the time of

exposure to be effective. However, sunscreens are easily rubbed off or washed off by sweating or swimming and can also be lost by penetration into the skin.

Another means to prevent skin deterioration or ageing, respectively, is to provide compounds scavenging free radicals. In this respect EP 0 761 214 discloses singlet oxygen quenchers comprising aniline derivatives and difurfuryl amine derivatives, which are reported to reduce the oxidative stress to the skin.

Yet, all these means and methods are not sufficiently capable to protect the skin from the growing challenge in our environment. To this contributes an increased atmospheric pollution and also social behaviour, according to which sun-tan is associated with health, beauty and status. As a consequence many people expose their skin to sun radiation to acquire a tan in spite of the negative results accompanying such behaviour being well known. This problem even gets more prominent with the ozone shield covering the earth becoming thinner, resulting in a heavier exposure of living beings to UV radiation.

Consequently there is a need in the art to provide a better protection of the skin to environmental factors, such as stress or sun radiation.

Accordingly, an object of the present invention is to obviate the drawbacks of the prior art and to provide such means in order to protect the skin from unfavourable influences encountered in the environment, in particular from oxidative or chemical stress or sun radiation.

This problem has been solved by providing a substance, that is capable to essentially modify, in particular decrease, inhibit or even block the endogenous CD_{1d} function in epithelial cells.

In the figures,

Fig. 1A. Wild-type mice exhibit skin damage (burning) following exposure to a single dose ($86mJ/m^2$) of UVB radiation.

Fig. 1B. Wild-type mice exhibit skin damage (burning) following exposure to a single dose ($86mJ/m^2$) of UVB radiation. (Close-up).

Fig. 1C. CD1d knockout mice show no obvious signs of skin damage following exposure to a single dose ($86mJ/m^2$) of UVB radiation.

Fig. 1D. CD1d knockout mice show no obvious signs of skin damage following exposure to a single dose ($86mJ/m^2$) of UVB radiation. (Close-up).

Fig. 2 Difference in degree of UVB-induced skin damage between wild-type (Right) and CD1d knockout (Left) mice exposed to two doses ($86mJ/m^2$) of UVB radiation.

Fig. 2A. Damaged (lesions) dorsal skin of wild-type mice exposed to two doses ($86mJ/m^2$) of UVB radiation (Close-up).

Fig. 2B. Undamaged dorsal skin of CD_{1d} knockout mice exposed to two doses ($86mJ/m^2$) of UVB radiation.

Fig.3. CD_{1d} knockout mice exhibit increased epidermal apoptosis in their dorsal epidermis compared to wild-type mice, as measured by TUNEL. Wild-type (A) and CD1d knockout (B) mouse skin not exposed to UV-irradiation. Wild-type (C) and CD_{1d} knockout (D) mouse skin 48h after a single exposure ($86mJ/m^2$) to UV-B radiation.

Fig. 4 a and b are graphs indicating the approximate amount of CD_{1d} in different tissues in mice and human.

Fig. 5 illustrates, based on in silico analysis, that the human and mouse CD_{1d} promotors are capable of being regulated by a number of transcription factors known to play a key role in inflammation and stress.

The present invention is essentially based on the finding that CD_{1d}, a transmembrane protein expressed by a number of different cells, in particular epithelial cells, modulates a variety of different responses of the cell to stress. As will become evident from the following detailed description of the preferred embodiments, essentially modifying, specifically decreasing /inhibiting/blocking the endogenous CD_{1d} function in cells bearing said membrane molecule allows to prevent the detrimental effects of stress, including ultraviolet radiation-induced skin damage, e.g. as a result of burning, epidermal hyperplasia, mutant p53 accumulation, inflammation, immune suppression and skin ageing. Even more surprising is the finding that when essentially decreasing/inhibiting/blocking CD_{1d} function in epithelial cells induction of cancer in said cells, i.e. basal cell carcinoma, squamous cell carcinoma, malignant melanoma, colon, breast, liver, prostate, kidney, pancreas cancer etc., may be prevented. In addition, surprisingly it has been found that modifying, in particular decreasing/inhibiting/blocking CD_{1d} function influences hair growth and/or development.

CD_{1d} as such is a type 1 transmembrane MHC class 1 like protein that non-covalently associates with β_2-microglobulin. The CD_{1d} molecule is recognized by a T-cell receptor of natural killer T-cells (NKT) which play a role in immune modulatory and effectory reactions. It has been demonstrated that CD_{1d} may present lipids to NKT cells for their activation, which notion is supported by the CD_{1d} crystal structure having two highly hydrophobic grooves, necessary for presenting hydrophobic molecules such as lipids to the immune system.

Without wishing to be bound to any theory it is currently assumed that one of the endogenous tasks of CD_{1d} in living organisms is to directly control normal epithelial cell homeostasis. Normal skin homeostasis is dependent on the critical and fine tuned balance between epidermal differentiation, apoptosis, proliferation and anti-apoptosis of epidermal cells. In the skin, these processes are regulated via lipids, in particular by means of ceramides and glucosylceramides (sphingolipids). While the nucleated cell layers generate glucosylceramides (GlcCer), the proportions of GlcCer to Cer decrease late in epidermal differentiation, with the Cer content peaking in the stratum corneum acting as extracellular constituents of the epidermal permeability barrier. In addition to their structural properties, ceramides are

associated with inhibition of cellular proliferation, induction of cellular differentiation and programmed cell death. In contrast, GlcCer induce cell proliferation and inhibit programmed cell death.

Based on the findings in the present invention, CD_{1d} appears to be one of the receptors via which the above mentioned lipids might fulfil their biological task. Specifically, CD_{1d} seems to negatively regulate apoptosis. In consequence, in cells under a stress situation, e.g. when exposed to UV-radiation, CD_{1d} supports a continued existence of said stressed cells, even when their genetic material is damaged and/or mutated, which damaged cells will contribute to inflammation processes induced and eventually account for the phenomenon of ageing or eventual tumour development.

In decreasing/inhibiting/blocking and/or modifying endogenous CD_{1d} function, apoptosis of cells under stress may be promoted, instead of their survival and propagation, with the effect that cells that have been damaged to a certain extent, particularly at the DNA level, do not have the chance to proliferate and in case disseminate in the body. The cells once dead will then be extinguished by natural processes in the body and be replaced by "healthy" epithelial cells. Likewise, by means of decreasing/inhibiting/blocking or modifying CD_{1d} also an interaction with NKT is substantially prevented or altered, wherein the phenomenon of immune suppression during exposure to UV radiation will be essentially reduced or barred at all. Also, this condition is supposed to assist the organism's immune system to eradicate damaged cells, brought about by exposure to UV.

The substance capable of decreasing/inhibiting/blocking and/or modifying the CD_{1d} trans-membrane molecule's activity may be any substance interfering with the endogenous biological function of CD_{1d}, and in particular preventing or reducing association of CD_{1d} with endogenous or exogenous lipids. The substances are obtainable by a process comprising the steps of (a) exposing epithelial cells to a substance of interest, (b) subjecting the epithelial cells to a stress situation, (c) determining the effect of said stress to said epithelial cells by screening for one or more of the following assays: (i) epithelial hyperplasia (H&E), (ii) epithelial proliferation (BrUd, PCNA), (iii) epithelial apoptosis, (iv) p53 mutation

accumulation, (v) quantitative and qualitative assessment of epithelial lipids, (vi) co-clustering patterns of apoptotic and non-apoptotic cell surface receptors, (vii) production of pro-inflammatory cytokines, (viii) production of immuno-modulatory cytokines, (ix) markers of inflammation, (x) anti-apoptotic transcription factor activity (xi) markers of ageing, and (d) comparing the results obtained with a control. Such a control may e.g. be an assay, wherein the cells have been subjected to the same stress situation, wherein, however, no substance to be investigated had been added (negative control) or cells lacking CD_{1d} had been subjected to the stress situation (positive control). Likewise a control may also be, including a substance with a known positive effect in the assay (i.e. a substance preventing stress symptoms) and determining the difference in effect achieved by the substance investigated and the known substance (positive control).

A substance is considered to be active in the context of this application, in case it prevents the negative effects of stress as detailed according to any of the above assays.

It will be appreciated that CD_{1d} activity may be decreased/inhibited/blocked and/or modified by substances acting on the genetic level or at the protein level.

Substances acting on the genetic level are compounds influencing, in particular preventing transcription or translation of the CD_{1d} gene, such as polynucleotides anti-sense to at least a part of the CD_{1d} gene or the CD_{1d}-mRNA.

The terms oligonucleotide and polynucleotide, which are interchangeably used herein, include linear oligomers/polymers of natural or modified monomers or linkages, including desoxyribo-nucleosides, ribonucleosides, α-anomeric forms thereof, polyamide nucleic acids, and the like, capable of specifically binding to the target nucleic acid by way of a regular pattern of monomer-to-monomer interactions, such as Watson-Crick type of base pairing, Hoogsteen or reverse Hoogsteen types of base pairing, or the like. Usually the monomers are linked by phosphodiester bonds or analogs thereof to form oligonucleotides ranging in size from a few monomeric units, e.g. 3-5, to several 100 or even thousands of monomeric units.

The (anti-)sense oligo-/polynucleotides may also contain pendent groups or moieties, to enhance specificity, nuclease resistance, delivery, or other property related to efficacy, such as e.g. cholesterol moieties, duplex intercalators such as acridine, poly-L-lysine, "end capping" with one or more nuclease-resistant linkage groups such as phosphorothioate, and the like. The corresponding oligonucleotide may be used for decreasing/inhibiting/blocking transcription, RNA processing and/or translation of the mRNA, Consequently, the oligonucleotide may comprise exon, but also intron sequences of the CD_{1d} /target gene, as desired.

The nucleotide sequence of the human CD_{1d} gene or mRNA is obtainable from NCBI (Accession numbers: X14974 and NM_001766, respectively). Based on his general knowledge and skill, the skilled person may select at least a portion of the coding region of the CD_{1d} gene and design an appropriate anti-sense polynucleotide, that prevents transcription and/or translation of the CD_{1d} gene. Likewise, also a part of the non-coding region of the CD_{1d} gene may serve as an agent for preventing transcription or reducing the number of transcripts, respectively, of the CD_{1d} gene. Here, in particular parts of the promotor region may serve as a template for preparing an antisense polynucleotide, but likewise transitions regions from introns and exons and vice versa. According to a preferred embodiment such a substance may be an DNA or a cRNA (RNA-interference).

Yet, apart from the CD_{1d} gene being the target, also the activity of a number of regulatory molecules which control epithelial homeostasis such as ceramides and/or glucosylceramides, may be modified such, that they exert the desired effect on the CD_{1d} molecule. To this end, the number of the glucosylceramide synthase transcripts may be reduced by designing an polynucleotide antisense to at least a part of the glucosylceramide synthase gene or glucosylceramide synthase mRNA, so that eventually the signal to epithelial cells to proliferate is turned down. The nucleotide sequence of the glucosylceramide synthase gene is disclosed in Ichikawa et al., PNAS 93 (1996), 4638-4643, which document is incorporated herein by way of reference. Likewise, non coding regions may serve as a template for the antisense polynucleotide, such as the promotor region and/or transitions from introns to exons and vice versa. According to a preferred embodiment such a substance may be a DNA or a cRNA (RNA-interference).

Apart from reducing the proliferation signal also the signal driving epithelial cells to apoptosis via the CD_{1d} molecule may be enhanced. In this respect the number of corresponding transcripts may be increased, which may be effected by providing a higher number of polynucleotides encoding a sequence comprised by the sphingomyelinase or ceramide synthase gene and/or the sphingomyelinase or ceramide synthase mRNA.

Apart from the genetic level, the biological activity of the CD_{1d} molecule may also be modified, in particular decreased/inhibited/blocked at the protein level, in particular by any substance binding to the CD_{1d} receptor on or in epithelial cells and decreasing/inhibiting/blocking the endogenous biological functionality thereof.

According to a preferred embodiment the substance capable of modifying, in particular decreasing/inhibiting/blocking biological CD_{1d} function is a polypeptide or a peptide, in particular hydrophobic peptides, more preferably an antibody, or a part thereof, that binds to the CD_{1d} receptor and decreases/inhibits/blocks its biological function, such as the interaction with NKT. As parts thereof, in particular mini-antibodies are envisaged lacking the F_c-part. According to an alternative embodiment the substance capable of decreasing/inhibiting/ blocking the biological CD_{1d} function may also be a soluble CD_{1d} receptor, that is, that part of the polypeptide lacking the region, anchoring the polypeptide in the membrane. The soluble CD_{1d} receptor will scavenge the in vivo ligands that promote survival of the stressed cells, thus promoting apoptosis. In addition, binding of the natural killer cells to CD_{1d} in vivo will be reduced, thus preventing activation of the T-cells and consequently inflammatory and/or immunosuppressive reactions.

According to a preferred embodiment the substance capable of decreasing/inhibiting/ blocking and/or modifying biological CD_{1d} function is a lipid derived from a plant, microbe or animal, including a phospholipid, ganglioside, sphingolipid, glycosphingolipid, phosphatidylinositol phosphate, sterol, glyceride or fatty acids. These lipids may influence CD_{1d} function by directly binding the CD_{1d} molecule or indirectly by influencing CD_{1d} gene expression.

According to another embodiment the substance capable of stimulating and/or modifying biological CD_{1d} function is a phenol, polyphenol or a substance derived or obtainable from Ginkgo. These molecules may influence CD_{1d} function by directly binding the CD_{1d} molecule or indirectly by influencing CD_{1d} gene expression.

According to an alternative embodiment the substance capable of decreasing/inhibiting/ blocking and/or modifying biological CD_{1d} function is a ceramide, such as ceramide 8 or sphingosylphosphorylcholine (SPC) or a ligand of a receptor belonging to the TNF-superfamily, in particular CD95/APO-1/Fas, which induces apoptosis thus interfering with the anti-apoptotic function of CD_{1d}. In another embodiment the objective substance is an organic compound obtained by chemical synthesis.

It is well established that ceramide glycosylation, via glucosylceramide synthase, and the subsequent build up of glucosylceramides allows cellular escape from stress-induced programmed cell death, conferring cancer cell resistance of a variety of cancers including breast, skin, colon and epitheliod carcinomas, to cytotoxic anti-cancer agents. As CD_{1d} can bind glucosylceramide and is over-expressed by the same multi-drug-resistant cancer cells (e.g squamous cell carcinoma), it is envisioned that the anti-apoptotic activity of CD_{1d} regulates cancer cell resistance to cytotoxic drugs, possibly at the level of protein-glucosyl-ceramide binding. Thus, in principle the substances of the present invention that decrease/inhibit/block and/or modify endogenous CD_{1d} function strongly decrease multi-drug resistance of a variety of cancers including skin, gut and breast cancers.

In principle, the substances of the present invention may also influence the bi-directional trafficking of CD_{1d} to and from the membrane.

The substances may be included in any composition suitable for administering the substance to an individual, in particular a food composition, a cosmetic composition or a pharmaceutical composition.

The pharmaceutical compositions containing at least one of the substances capable of decreasing/inhibiting/blocking or modifying the CD_{1d} surface molecule according to the invention can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, compositions are administered to a patient already suffering from a disease, as described herein under, in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as "a therapeutically effective dose". Amounts effective for this will depend on the severity of the disease and the weight and general state of the patient.

In prophylactic applications, compositions containing at least one of the substances capable of decreasing/inhibiting/blocking or modifying the CD_{1d} surface molecule according to the invention are administered to a patient susceptible to or otherwise at risk of a particular disease. Such an amount is defined to be "a prophylactic effective dose". In this use, the precise amounts again depend on the patient's state of health and weight.

The compounds of the invention are preferably administered with a pharmaceutical acceptable carrier, the nature of the carrier differing with the mode of administration, for example parenteral, intravenous, oral and topical (including ophthalmic) routes.

The desired formulation can be made using a variety of excipients including, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin cellulose, magnesium carbonate. This composition may be a tablet, a capsule, a pill, a solution, a suspension, a syrup, a dried oral supplement, a wet oral supplement, dry tube-feeding, wet tube-feeding etc.. In order to control the drug release, sustained-release formulations can also be used.

The kind of the carrier/excipient and the amount thereof will depend on the nature of the substance and the mode of drug delivery and/or administration contemplated. E.g., for formulations containing weakly soluble antisense oligonucleotides, micro-emulsions may be employed, for example by using a non-ionic surfactant such as Tween 80 in an amount of about 0.04-0.05% (w/v), to increase solubility. Other components may include antioxidants, such as ascorbic acid, hydrophilic polymers, such as, monosaccharides, disaccharides, and

other carbohydrates including cellulose or its derivatives, dextrins, chelating agents, such as EDTA, and like components well known to those in the pharmaceutical sciences. These various components utilized provide a variety of functions, including regulation of drug concentration, regulation of solubility, chemical stabilization, regulation of viscosity, absorption enhancement, regulation of pH, and the like. For example, in water soluble formulations the pharmaceutical composition preferably includes a buffer such as a phosphate buffer, or other organic acid salts, preferably at a pH of between about 7 and 8.

It will be appreciated that the skilled person will, based on his own knowledge select the appropriate components and galenic form to target the active compound to the tissue of interest, e.g. the colon, stomach, skin, kidney or liver, taking into account the route of administration which may be by way of injection, topical application, intranasal administration, administration by implanted or transdermal sustained release systems, and the like.

The objective substance may also be formulated in a cosmetic product, such as lotions, shampoos, creams, sun-screens, after-sun creams, sun-blocker, anti-ageing creams, ointments and/or anti-hair loss liquids. This proves in particular advantageous for essentially decreasing/inhibiting/blocking CD_{1d} function in the skin and to prevent the adverse effect of sun radiation, photo-ageing and exposure of the skin to free radicals. Thus, e.g. by providing a sun-screen containing in addition to a common agent, absorbing UV-light a substance as defined herein, a protection to the sun may be provided, which by far exceeds anything known so far. This feature is based in particular on the fact that the objective substance will penetrate the skin and exert its effect after having reached the target molecules. Since this effect will stay for a while, protection to the sun will even be present in case the sun-screen has been rubbed off or has been washed off, as e.g. during sport etc.. Yet, apart from sun-screens the objective substances may be included in common day-creams, lotions etc. to prevent negative effects of the daily environment, including pollution, oxidative stress etc.. It will be appreciated that the present cosmetic products will contain a mixture of different ingredients known to the skilled person, ensuring a fast penetration of the objective substance into the skin and preventing degradation thereof during storage.

Another high important composition according to the present invention is food material. In our present society a great deal of food is ingested, such as sausages, salted or grilled meat etc., that contains preservatives, ingredients or substances, that are injurious to the gut. E.g. grilled meat contains aliphatic and aromatic compounds known to be cancerogenic. Also preservatives, that kill micro-organisms contained in food material (e.g. sausages) by manipulating their DNA, will exert a similar effect to cells of the gut. In fact, the number of intestinal cancer is steadily increasing in our society, which may be attributed at least in part to the type of food taken by humans.

Consequently, the present invention provides a food composition that prevents the onset and/or development of such gut disorders, such as a composition selected from the group consisting of milk, or fermented milk products, such as e.g. yogurt, curd, cheese, milk based fermented products, ice-creams, milk based powders, infant formulae, cereal products and fermented cereal based products, mineral water, chocolate or pet food containing at least a substance capable of essentially decreasing/inhibiting/blocking and/or modifying CD_{1d} function. Since the objective compound will be contained in a food material in amounts, that do not affect the original taste thereof, the consumer will not notice any change in the product, but will experience the beneficial effects thereof, namely a protective or even curing effect. Once the food material has been ingested the objective substances will arrive at the target cells, which may be epithelial cells of the gut, i.e. of the stomach or the intestine, and will bind to the CD_{1d} receptor and exert its activity. As a consequence, cells, that are already damaged will preferably go to apoptosis instead of being maintained in said damaged form.

Since epithelial cells bearing CD_{1d} have been found in a number of organs, such as the liver, the small intestine, the colon, the kidney, the prostate, the uterus, the pancreas, breast, skin and conjunctivia, the choice of the composition as detailed above will, by and large depend on the target tissue. As will be understood, for skin a cosmetic product might be the composition of choice, while in case of delivering the objective substance directly to the gut or the colon, a food product may be first choice. However, a food product may also be suitable for delivering the objective substance or substances to other organs, such as the kidney or the liver, which will

depend on the stability of the substance in the body and its capacity of being absorbed by the body in the gut. Since food is a daily ingested material such a product offers a great variety of different possibilities. Yet, in case the objective substance is prone to degradation in the gut a pharmaceutical composition may be selected, providing e.g. encapsulation or other galenic forms to deliver the objective substance to the target tissue/to target cells.

It will be understood that the concept of the present invention may likewise be applied as an adjuvant therapy assisting in presently used medications. In this respect the pharmaceutical composition of the present invention may be administered together with e.g. cytostatika so as to prevent escape of the tumor treated from the treatment, which sometimes occurs in long term treatments of certain tumors or to assist in killing residual cancer cells not captured with the pharmaceutical regimen. Since the substance(s) of the present invention may easily be administered together with food material special clinical food may be applied containing a high amount of the objective substances. Also melanoma may be directly treated with an antibody medication against melanoma together with a pharmaceutical composition or a cosmetic product as described herein. It will be clear that on reading the present specification together with the appending claims the skilled person will envisage a variety of different alternatives to the specific embodiments mentioned herein.

In principle, the substances according to the present invention may be used for the treatment and/ or prevention of damages in epithelial tissues, such as e.g. in the skin, gut, eye, lung, liver, prostate, breast, kidney and/or in the uterus, which are produced by a stress situation, e.g. by means of a chemical, biological or a physical stress, e.g. by exposure to oxidants or carcinogens, exposure to bacteria, viruses, fungi, lipids derived from surrounding cells and/or microbes, or exposure to UV-irradiation. Likewise, the substances may be utilized for preventing and/or treating hair loss.

Consequently, the substances and/or compositions according to the present invention may be utilized for treating and or preventing damages of the skin, in particular actinic and ageing damages of the skin such as dryness, actinic keratoses, irregular pigmentation (notably comprising freckling, lentigines, guttate hypomelanosis and persitent hyperpigmentation),

wrinckling (notably comprising fine surface lines and deep furrows), stellate pseudoscars, elastosis, inelasticity, telangiectasia, venous lakes, purpura, comedones, sebaceous hyperplasia, acrochordon, cherry angiogema, seborrhea keratosis, lentigo, basal cell carcinoma and squamous cell carcinoma, skin burning and/or blistering, cataract formation, epidermal hyperplasia, inflammation, immune suppression, and cancer, e.g. non-melanoma and melanoma skin cancers.

In order to arrive at additional substances having the above characteristics the present invention also provides a method for screening for such substances. In this method epithelial cells are utilized that may be in the form of a primary culture, i.e. directly derived from an individual or in the form of a cell line. For carrying out the method a cell culture is particularly preferred, since it allows for the continuous supply of epithelial cells during the experiments. Care must be taken that the cell culture of epithelial cells used exhibit the same phenotypic traits as do cells of a primary culture or epithelial cells directly obtained from a tissue sample. It will be understood that the person skilled in the art will select the starting material depending on the assay. Hence, if a first round assay is to be carried out a cell culture design seems to be most appropriate, while in case for further rounds, i.e. assessing the activity of potential candidates, the tissue or even the animal model seems to be more appropriate.

The epithelial cells are exposed to a substance of interest for a time period sufficient to ensure a contact of the substance with the cells. In a next step the epithelial cells are exposed to a stress situation, which may be effected e.g. by irradiating the cells with different dosages of UV light, or adding hydrogen peroxide or toxic chemicals to the cell culture. However, the type of stress is not critical as long as the cells are challenged to initiate processes, normally started under stress situations, such as e.g. the production of pro-inflammatory cytokines e.g. IFN- γ, TNF-α, IL-1, IL-6, IL-8, apoptosis, altered lipid metabolism, increased production of p53, altered cell signaling as a result of altered patterns of cell surface receptor co-clustering, NF-κB activation, AP1 activation, showing hyperproliferation (anti-apoptosis), altered barrier function etc.. It will be understood that also more than one substance may be tested at the same time, that is a cocktail of one or more substances, which might prove beneficial for the second or further round of assaying.

In a next step the effect of said stress on the epithelial cells is determined by assessing one or more of the following features, for example: epithelial proliferation (PCNA: Ouhtit et al., American Journal of Pathology [2000], 156: 201-207; BrUd: Lu Y-P et al., Cancer Research [1999], 59: 4591-4602); epithelial apoptosis (Tunel Assay; modification of protocol outlined by Ouhtit et al., American Journal of Pathology [2000], 156: 201-207); p53 mutation accumulation (Allele-specific polymerase chain reaction [AS-PCR] and single-strand conformation polymorphism [SSCP], Ananthaswamy et al., Nature Medicine [1997], 3: 510-514); production of pro-inflammatory and immuno-modulatory cytokines (e.g. TNF-α, PGE-2, IL-1, IL-6, IL-8, IL-4, IL-10, Platelet Activating Factor (PAF), TGFβ); markers of inflammation (e.g. Cox-2, iNos); and anti-apoptotic transcription factor (including AP-1, NFkappaB) activity by TaqMan Real-time RT-PCR, ELISA and Immunohistochemistry; qualitative and quantitative assessment of phospholipids, glycosphingolipid and sphingolipid content (Electron-Spray Tandem Mass Spectrometry); analysis of co-clustering patterns of epithelial cell surface receptor molecules including cytokine receptors (e.g. IL-6), molecules of the TNF-superfamily of receptors (e.g. CD95/APO-1/Fas) and growth regulating receptors (e.g. EGF, Insulin) by fluorescence resonance electron transfer analysis (FRET); markers of ageing, e.g. elastases, collagenases, metalloproteinases, gelatinase, stromelysins, telomerase.

The results obtained are then compared with a control, which may simply be an assay, wherein the same type of cells are exposed to the same stress conditions with the proviso, that no compound to be assessed for its CD_{1d} decreasing/inhibiting/blocking capacity is provided (negative control). A positive control is represented by a $CD_{1d}^{-/-}$ animal/cell line exposed to the stress situation, wherein CD_{1d} activity is lacking at all. Likewise, a control may also be, including a substance with a known positive effect in the assay (i.e. a substance preventing stress symptoms) and determining the difference in effect achieved by the substance investigated and the known substance (positive control).

The present invention also pertains to using the findings of the present invention in a gene reporter assay for screening substances stimulating CD_{1d} promoter activity.

The following examples illustrate the invention in more detail without restricting the same thereto.

Example 1

Generation of CD_{1d} mutant mice

Mouse CD_{1d} is encoded by two genes, CD_{1d1} and CD_{1d2}, that share a high degree of nucleotide sequence identity (Bradbury et al., EMBO J., 7 (1988), 3081-3086). The product of the CD_{1d1} gene is recognized by all anti-CD_1 antibodies that have been described, whereas surface expression of the CD_{1d2} product has not yet been demonstrated. In addition, the predicted α2 domain of the CD_{1d2} gene product lacks an intra-domain disulfide bond that is found in the α2 domain of all published classic and non-classic MHC class I molecules (Bradbury, supra). This disulphide bond is thought to be critical for the folding of the antigen-binding groove. Thus, the CD_{1d2} gene may not encode a functional antigen-presenting molecule, and all functions previously attributed to mouse CD_1 may be effected by the product of the CD_{1d1} gene. For this reason, it was decided to introduce a targeted mutation into the CD_{1d1} gene, while leaving CD_{1d2} intact.

The CD_{1d} gene was isolated from a strain 129/Sv phage library with a probe generated by polymerase chain reaction. The targeting construct was prepared using a 2.8 kb ApaI fragment containing the 5' region of the CD_{1d} gene, a 3.2 kb BamHI-NotI fragment containing the 3' region of the CD_{1d} gene (the NotI site in this fragment comes from the pBluescript vector into which phage DNA was initially subcloned), a neomycin resistance gene (neo), and the pBluescript plasmid (Stratagene). This construct was designed to delete a fragment of about 200 bp from the exon encoding the α2 domain of CD1d1. The strain 129/Sv-derived embryonic stem (ES) cell line TL1 was transfected with the NotI-linearized targeting vector. G418-resistant colonies were selected and isolated as described in Van Kaer et al., Cell 71 (1992), 1205-1214. Genomic DNA from individual clones was digested with EcoRI and hybridized with a 2.3 kb ClaI-EcoRI probe from the 5' end of the CD1d1 gene. Recombination was confirmed by digestion with KpnI and hybridization with a 700 bp BamHI-EcoRI probe from the 3' end of the CD_{1d1} gene. Chimeric mice were mated with

C57BL/6 mice to score for germline transmission, and heterozygous mutant mice were intercrossed to obtain (C57BL/6x129/Sv) F2 homozygous mutants. Mice were typed for their CD1d1 status by genomic southern blotting with the 5' probe. Mutant mice were healthy and bred normally.

Because the ES cells and mouse strain used to generate mutant animals differ in their TL status (129/Sv is a TL+ stain and C57BL/6 is a TL- strain) all mice used in this study were genotyped for TL. To type mice for their TL status, tail DNA was digested with BglII and hybridized with a TL-specific probe that detects a polymorphism between strains 129/Sv (TL+) and C57BL/6 (TL-) (Pontarotti et al., Proc. Natl. Acad. Sci. USA 83 (1986), 1782-1786). This probe was generated by polymerase chain reaction using a set of primers designed on the basis of published sequences (Pontarotti, supra):

5'-TATACAGAGCTCCGTAGGAC-3' ; and

5'-AGTTGTCTGCAGCCACGAAC-3'.

The CD_{1d1} mutant and wild-type mice were housed in a specific-pathogen-free barrier animal facility, accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). Animals were used between 12-16 weeks of age at the start of the experiments. They were housed in filter-protected cages with a 12h light-dark controlled cycle, and provided with autoclaved NIH open formula mouse chow and water ad libidum. The institutional Animal Care and Use Committee approved all procedures. Within each experiment all mice were aged- and sex-matched.

It is underlined that other genetic backgrounds can be used for creating a CD_{1d} mutant mouse, such as Balb/C genetic background.

Example 2

UV Irradiation of mice

A bank of five Philips TL-40W/12 sunlamps (Philips, The Netherlands) was used to irradiate the mice. These lamps emit a spectrum from 270 to 400 nm; 54% of the irradiation was within the UVB range (280-315 nm) of the solar spectrum, with 45% being in the UVA (315-

400nm) region and less than 1% in the UV-C (240-280 nm) range. The irradiance of the five bulbs averaged 10 W/m^2, as measured by a UVB PMA research radiometer.

The dorsal hair of the mice was removed with electric clippers and the mice were placed into a plexiglass box separated into individual compartments by Plexiglas dividers and covered with a wire top which decreased the incident dose by 14%. For each UV-irradiation, the box was placed each time in the same position under the lamps to compensate for the uneven distribution of energy along the length of the bulbs. The mice were exposed once or twice to an incident dose of 86 mJ/cm^2 UVB from five Philips TL-40W/12 sunlamps. Mice were exposed to a second dose of UVB radiation 96h after the first exposure. All mice were analyzed for signs of skin damage 24, 48, 72 and 96 h after their last UVB exposure.

Visually, a clear difference in the degree of skin damage was observed between wild-type and CD1d knockout mice following UVB-irradiation of their shaved dorsums. Whilst clear and significant skin damage (burning, skin lesions) was exhibited by UV-irradiated wild-type mice, no obvious signs of skin damage were detected in UV-irradiated CD1d knockout mice.

Example 3

Measurement of apoptosis in epidermis

Apoptotic cell death was detected using the DeadEnd™ Fluorometric TUNEL System (Promega) which measures the fragmented DNA of apoptotic cells by catalytically incorporating fluorescein-12-dUTP at 3' –OH DNA ends using the enzyme Terminal Deoxynucleotidyl Transferase (TdT). TdT forms a polymeric tail using the principle of the TUNEL assay. Briefly, formaline fixed paraffin embedded tissue sections on slides were deparaffinized twice in fresh xylene for 5 min at room temperature. They were washed in 100% ethanol for 5 min and then rehydrated sequentially by immersing the sections through graded (100%, 95%, 85%, 70%, 50%) ethanol washes for 3 min. Afterwards, the sections were immersed in 0.85% NaCl for 5min, washed in PBS and then fixed in 4% paraformaldehyde for 15 min followed by two washes in PBS. After removing residual fluid from the sections by tapping, each tissue section was covered with 20μg/ml proteinase K

(Sigma) for 8-10 min at room temperature. After proteinase K treatment, tissue sections were rinsed in PBS and then fixed by immersing in 4% paraformaldehyde for 5 min. This was followed by a wash in PBS, removal of residual fluid by tapping and incubation of the sections in equilibrium buffer (Promega) for 5-10 min. After equilibration, the sections were incubated in a humidified chamber with TdT enzyme for 1 h at 37°C. Sections were soaked in stop buffer (SSC; Promega) for 15 min to terminate the reactions and then rinsed in three changes of PBS. After rinsing, sections were stained with propidium iodide solution freshly diluted to 1μg/ml in PBS for 15 min in the dark. They were then washed three times in deionized water for 5 min, and afterwards, excess fluid wiped off the area surrounding the cells. The sections were then immediately examined under a fluorescence microscope.

At 2, 6, 24, 48, 72 and 96h after UV exposure (acute/chronic) a TUNEL Assay (modification of protocol outlined by Ouhtit et al., American Journal of Pathology [2000], 156: 201-207), of the skin taken from $CD_{1d}^{-/-}$ and wild-type mice was carried out. The results revealed that epidermal cells within the skin of $CD_{1d}^{-/-}$ mice were undergoing a high degree of apoptosis compared to wild-type mice. In contrast, in wild-type skin the epidermal cells were undergoing significantly less apoptosis.

Example 4

Measurement of Epidermal Hyperplasia

Dorsal skin biopsies were fixed overnight in 4% paraformaldehyde and paraffin embedded. Sections were stained with hematoxylin and eosin (H&E) and viewed by light microscopy.

Regardless of acute or chronic UVB exposure, $CD_{1d}^{-/-}$ mice exhibited significantly reduced epidermal hyperplasia 24, 48 or 72h after the last UVB treatment compared to UV-irradiated wild-type mice.

Example 5

Gene profiling

In order to elucidate CD_{1d} function a gene profiling assay comparing wild-type and CD_{1d} knockout mouse gene expression had been performed.

Skin tissue was extracted from 5 individual wild-type and CD_{1d} knockout mice and extracted separately using Trizol kit (Invitrogen AG, Basel, Switzerland) and then Qiagen RNeasy mini-kits (Basel Switzerland) according to manufacturer instructions with DNase I treatment to remove any genomic DNA contamination. RNA samples were quantified by OD then analyzed via dynamic gel electrophoresis with the Agilent Bioanalyser for intact 28S and 18S rRNA (All 28 / 18 ratio's were between 1.6 and 2.0). Study samples were judged to contain sufficient amounts of high-quality RNA for hybridization to GeneChips. As another quality control measure, prior to hybridization with Affymetrix GeneChips (Affymetrix, Inc., Santa Clara, CA), we confirmed that all samples gave strong signals for pre-selected genes, using Affymetrix test chips (Test chip 5' / 3' ratios were less than 3.0).

For skin, 10 μg total RNA was the starting material for all individual mouse samples. In general, total RNA was converted to biotinylated cRNA, hybridized in the Affymetrix probe array cartridge, stained, and then quantified. First and second strand cDNA synthesis was performed using the SuperScript Choice System (Invitrogen AG, Basel, Switzerland), according to manufacturer instructions, but using an oligo-dT primer containing a T7 RNA polymerase binding site. Labeled cRNA was prepared with the RNA Transcript Labeling kit (Enzo Biochem Inc., NY). Biotinylated CTP and UTP were used together with unlabeled NTPs in the reaction, and unincorporated nucleotides were removed with Nucleospin columns (Macherey-Nagel, Düren, Germany) .

cRNA (20 μg) was fragmented at 94 °C for 35 min in buffer containing 200 mM Tris-acetate pH 8.1, 500mM KOAc, 150 mM MgOAc. Prior to hybridization, fragmented cRNA in hybridization mix (Buffer containing 100 mM MES, 1M NaCl, 20 mM EDTA, 0.01%

Tween 20, 0.5 ng/µl BSA, 0.1 ng/µl herring sperm and Affymetrix controls), was heated to 95 °C for 5 min, cooled to 45 °C and loaded onto an Affymetrix probe array cartridge. The probe array was incubated for 16 h at 45 °C at constant rotation (60 rpm), then exposed to Affymetrix washing and staining protocol.

This protocol included:

- one wash with non-stringent buffer (6X SSPE, 0.01% Tween 20, 0,005% antifoam)
- one wash with stringent buffer (100 mM MES, 0.1 M NaCl, 0.01 % tween 20)
- First stain with 0.01 mg/ml streptavidin-phycoerythrin conjugate (Molecular Probes) in buffer containing 100 mM MES, 1M NaCl, 0.05% Tween 20, 4 mg/ml of BSA.
- one wash with non-stringent buffer (6X SSPE, 0.01% Tween 20, 0,005% antifoam)
- Second stain with 3 µg/ml of biotinylated anti-streptavidin + 0.2 mg/ml of IgG in buffer containing 100 mM MES, 1M NaCl, 0.05% Tween 20, 4 mg/ml of BSA.
- Third stain with 0.01 mg/ml streptavidin-phycoerythrin conjugate (Molecular Probes) in buffer containing 100 mM MES, 1M NaCl, 0.05% Tween 20, 4 mg/ml of BSA.
- one wash with non-stringent buffer (6X SSPE, 0.01% Tween 20, 0,005% antifoam)

A mathematical method was developed and applied to the raw GeneChip data for the selection of differentially regulated genes. This method moves beyond setting a single fold change cut-off by considering the standard deviation (SD) in the context of absolute expression, or absolute difference intensity (ADI).

The method included the following steps: (A) data processing by the commercially available "MAS5" Affymetrix program (Santa Clara, CA, USA) and rescaling, (B) logarithmic transformation to distribution normality of the rescaled data, (C) multiple hypotheses (one per gene) analysis of variance (ANOVA) testing, (D) the determination of the robust mean within condition SD (equation 1), within bins of 200 genes ordered by mean ADI levels, to determine a significance limit SD between condition, named the REGExpress function (equation 2 from Genome Biology 2001 2(12): preprint0009.1-0009.31); and (E) subsequent ranking of genes by the p value of the REGExpress and ANOVA, to help focus at effect

importance. The selection is made with the p value resulting from multiple hypotheses (one per gene) ANOVA testing and/or with the p value resulting from REGExpress.

Probe arrays were scanned at 488 nm using an Argon-ion Laser (made for Affymetrix by Agilent). Readings from the quantitative scanning were analyzed with Affymetrix Gene Expression Analysis Software.

The findings are summarized in the tables I to III below. The fold increase (+) or decrease (-) is the statistically significant relative fold increase or decrease of a gene expressed in CD1d knockout mice compared to the same gene expressed in wild-type mice. It becomes clearly evident that decreasing/inhibiting/blocking CD_{1d} upregulates genes controlling hair follicle development, and down-regulates genes involved in inflammation and cancer development.

Table I

Genes which regulate hair follicle development

Gene Name	Fold increase/decrease	Mean Wt	Mean CD1d -/-	Biological Function
mu-crystalline	+27.0	0.922	4.251	thyroid binding protein regulating hair follicle development
Patched homolog 2	+2.763	3.810	4.826	hair follicle development

Table II

Genes which regulate inflammation

Gene Name	Fold increase/ decrease	Mean Wt	Mean CD1d -/-	Biological Function	Disease Association
TGF beta activated Kinase	+1.743	3.630	4.186	Signalling molecule of the p38-MAPKinase and the Stress activated protein Kinase (SAPK) pathways.	
Rel-A (NFKappaB p65)	-0.735	7.350	7.043	anti-apoptotic, induction of inflammatory cytokines.	inflammatory disorders
cytochrome beta	-0.738	6.829	6.525	superoxide generation	

Plasminogen activator inhibitor (PAI-1)	- 0.357	3.906	2.875	Serine protease inhibitor. Regulates fibrolysis.	inflammatory disorders
MRP14	- 0.202	3.943	2.344	Ca^{++} dependent regulatory protein in inflammatory responses.	acute and chronic inflammatory responses e.g. Psoriasis
Mast cell protease	- 0.661	6.427	6.014	proteolysis and peptidolysis.	inflammation
P-Selectin	- 0.685	6.439	6.060	cell adhesion	inflammation
TFII-I	+1.373	5.707	6.024	Transcription factor which regulates c-Fos activity	
Interleukin-6	- 0.268	1.882	0.565	cytokine: multi-functional	inflammation

Table III

Genes which regulate cancer growth/development:

Gene Name	Fold increase/ decrease	Mean Wt	Mean CD1d -/-	Biological Function	Disease Association
Rel A (NFKappaB p65)	-0.735	7.350	7.043	oncogenic-transforms cells	cancer
Plasminogen activator inhibitor (PAI-1)	- 0.357	3.906	2.875	serine protease inhibitor	metastatic tumors
P-Selectin	- 0.685	6.439	6.060	adherence	facilitates tumor metastisis.
Cathepsin S	- 0.697	7.545	7.184	cysteine protease	malignancy
Proliferin	- 0.234	1.870	0.419	regulates angiogenesis	mouse fibrosarcomas
Interleukin-6	- 0.268	1.882	0.565		secreted by basal cell carcinomas and malignant melanomas.
CSF-1 receptor	- 0.750	7.196	6.909	Growth factor regulating cell proliferation.	Cancer

Example 6

Evaluation of the inflammatory response induced by a single topical administration of TPA

Phorbol-12-myristate-13-acetate (TPA) provided by Sigma Aldrich (L'Isle d'Abeau Chesnes BP701, 38297 Saint Quentin Fallavier, France) is dissolved in acetone at the dose of 0.01 % (W/V) and 20 µl of the solution is applied topically onto the internal face of the right ear of $CD_{1d}^{-/-}$ mice or wild-type mice in order to induce an acute inflammatory response.

The animals are maintained in individual cages with a standard pellet diet in an animal room with a 12-hour light-dark cycle. The facilities provide a filtered air with a temperature of 22 +/- 2 °C and a relative humidity of 55 +/- 10 %.

The inflammatory response is quantified 6 hours, 24 hours and 48 hours after application by measuring the ear oedema using a micrometer (« oditest » provided by Kroeplin Gmbh, Postfach 1255 D36372 Schlüchlern, Germany).

The oedema is calculated as follow :
(oedema = ear thickness of the treated group – ear thickness of the acetone group).
The mean value of $CD_{1d}^{-/-}$ group is compared to the mean value of the wild-type group using the Student's t-test.

Example 7

Evaluation of the inflammatory response induced by a single topical administration of arachidonic acid

Arachidonic acid (5-8-11-eicosatetraenoic acid) provided by Sigma Aldrich (L'Isle d'Abeau Chesnes BP701, 38297 Saint Quentin Fallavier, France) is dissolved in acetone at the concentration of 140nM and 25 µl of the solution is applied topically onto the internal face of the right ear of $CD_{1d}^{-/-}$ mice or wild-type mice in order to induce an acute inflammatory response.

The animals are maintained in individual cages with a standard pellet diet in an animal room with a 12-hour light-dark cycle. The facilities provide a filtered air with a temperature of 22 +/- 2 °C and a relative humidity of 55 +/- 10 %.

The inflammatory response is quantified 1 hour, 2 hours, and 4 hours after application by measuring the ear oedema using a micrometer (« oditest » provided by Kroeplin Gmbh, Postfach 1255 D36372 Schlüchlern, Germany).

The oedema is calculated as follow :
(oedema = ear thickness of the treated group – ear thickness of the acetone group).
The mean value of $CD_{1d}^{-/-}$ group is compared to the mean value of the wild-type group using the Student's t-test.

Example 8

Evaluation of the DTH (delayed-type hypersensivity) reaction induced by oxazolone

Oxazolone (4-ethyoxymethylene-2-phenyl-oxazol-5-one) provided by Sigma Aldrich (L'Isle d'Abeau Chesnes BP701, 38297 Saint Quentin Fallavier, France) is dissolved in acetone at the concentration of 1% (W/V) and 50 µl of the solution is applied once daily for 4 days on the abdominal skin of shaved $CD_{1d}^{-/-}$ mice or shaved wild-type mice.

4 days later the animals are challenged by a single administration (20µl) onto the internal face of the right ear of oxazolone dissolved in acetone at the dose of 0.3%. The post-challenge response is quantified 24 hours and 48 hours after application by measuring the ear oedema using a micrometer (« oditest » provided by Kroeplin Gmbh, Postfach 1255 D36372 Schlüchlern, Germany).

The oedema is calculated as follow :
(oedema = ear thickness of the treated group – ear thickness of the acetone group).
The mean value of $CD_{1d}^{-/-}$ group is compared to the mean value of the wild-type group using the Student's t-test.

Example 9

Evaluation of skin damages induced by UV irradiation using a solar simulator

A solar simulator (Oriel 81050) equiped with an UVC filter is used to irradiate $CD_{1d}^{-/-}$ mice or wild-type mice.

Irradiation : UVB + UVA doses and to be precised

Effect on epidermis : SBC counts, epidermal hyperplasia measurement

Effect on the dermis: MMP1 and MMP3 expression with immunohistochemical methods

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27. Aug. 2002

Claims

1. A substance capable to decrease/inhibit or modify endogenous CD_{1d} function, obtainable by a process comprising the steps of :

   (a) exposing epithelial cells to a substance of interest,

   (b) subjecting the epithelial cells to a stress situation,

   (c) determining the effect of said stress to said epithelial cells by screening for one or more of the following assays,

      (i) epithelial hyperplasia (H&E),

      (ii) epithelial proliferation (BrUd, PCNA),

      (iii) epithelial apoptosis (TUNEL),

      (iv) p53 mutation accumulation,

      (v) quantitative and qualitative assessment of epithelial lipids,

      (vi) co-clustering patterns of apoptotic and non-apoptotic cell surface receptors,

      (vii) production of pro-inflammatory cytokines,

      (viii) production of immuno-modulatory cytokines,

      (ix) markers of inflammation,

      (x) anti-apoptotic transcription factors,

      (xi) markers of ageing,

   (d) comparing the results obtained with a control,

   wherein a modification, decrease or inhibition of stress indicators as determined in c) is indicative for a substance capable of modifying and/or reducing/inhibiting endogenous CD_{1d} function.

2. The substance according to claim 1, which is capable of preventing and/or treating detrimental effects of stress to epithelial cells.

3. The substance according to claim 1, which is capable of preventing or treating hair loss.

4. The substance according to any of the preceding claims, which is a compound reducing/inhibiting the transcription and/or translation of the CD_{1d} gene.

5. The substance according to claim 1 to 4, which is a polynucleotide antisense to a sequence comprised by the CD_{1d}-gene and/or the CD_{1d}-mRNA.

6. The substance according to any of the claims 1 to 4, which is a polynucleotide antisense to a sequence comprised by the glucosylceramide synthase gene and/or the glucosyl-ceramide synthase mRNA.

7. The substance according to any of the claims 1 to 4, which is a polynucleotide sense to a sequence comprised by the sphingomyelinase or ceramide synthase gene and/or the sphingomyelinase or ceramide synthase mRNA.

8. The substance according to any of the claims 1 to 4, which is a polypeptide or peptide, binding to CD_{1d} and essentially reducing/inhibiting or modifying CD_{1d} function.

9. The substance according to claim 8, wherein the polypeptide is an antibody or the variable part of an antibody.

10. The substance according to any of the claims 1 to 4, which is a lipid.

11. The substance according to claim 10, wherein the lipid is a sphingolipid, glycosphingo-lipid, phospholipid, ganglioside, sterol, fatty acid, glyceride or phosphatidylinositol phosphate,

12. The substance according to claim 10 and 11 which is derived from plants, microbes or animals.

13. The substance according to claims 1 to 4, 8 or claims 10 to 12, which is a ceramide, such as ceramide 8 or sphingosylphosphorylcholine, or a ligand of a receptor belonging to the TNF-superfamily, in particular CD95/APO-1/Fas, or a phenol or polyphenol, particularly a natural or synthetic epigallocatechin-3-gallate like molecule, or a substance derived from Ginkgo.

14. The substance according to any of the preceding claims for the preparation of a carrier for the prevention and/or treatment of the detrimental effects of stress to epithelial cells and/or hair loss.

15. A composition, containing at least a substance according to any of the preceding claims.

16. A composition according to claim 15, which is a food composition, a cosmetic composition or a pharmaceutical composition.

17. The composition according to claim 16, which is milk, yogurt, curd, cheese, fermented milks, milk based fermented products, ice-creams, milk based powders, infant formulae, cereal products, fermented cereal based products, mineral water, chocolate or pet food, or lotions, shampoos, creams, sun-screens, after-sun creams, anti-ageing creams and/or ointments or tablets, liquid, dried oral supplement, wet oral supplement, dry tube-feeding or wet tube-feeding or an anti-cancer drug.

18. Use of a substance according to any of the claims 1 to 14 or a composition according to any of the claims to 15 to 17 for the prevention and/or treatment of damages in epithelial tissues produced by a stress situation and/or for the prevention and/or treatment of hair loss.

19. The use according to claim 18, wherein the stress situation is a chemical stress, a biological stress or a physical stress.

20. The use according to any of the claims 19, wherein the chemical stress is exerted by exposure to oxidants or carcinogens, or wherein the biological stress is exerted by exposure to bacteria, viruses, fungi, lipids derived from surrounding cells and/or microbes, or wherein the physical stress is exerted by exposure to UV-irradiation.

21. The use according to any of the claims 18 to 20, wherein the damage is skin burning and/or blistering, cataract formation, epidermal hyperplasia, cancer, inflammation, immune suppression, skin ageing.

22. The use according to any of the claims 18 to 21, wherein the epithelial cells are derived from the skin, gut, eye, lung, prostate, liver, breast, kidney and/or the uterus.

23. The use according to claim 21, wherein the cancer is breast cancer, colon cancer, prostate cancer, liver cancer, pancreatic cancer, kidney cancer, non-melanoma and melanoma skin cancers.

24. A method for identifying substances reducing/inhibiting or modifying CD_{1d} function which comprises the following steps:

(a) exposing epithelial cells to a substance of interest,

(b) subjecting the epithelial cells to a stress situation,

(c) determining the effect of said stress to said epithelial cells by screening for one or more of the following assays,

(i) epithelial hyperplasia (H&E),

(ii) epithelial proliferation (BrUd, PCNA),

(iii) epithelial apoptosis (TUNEL),

(iv) p53 mutation accumulation,

(v) quantitative and qualitative assessment of epithelial lipids,

(vi) co-clustering patterns of apoptotic and non-apoptotic cell surface receptors,

(vii) production of pro-inflammatory cytokines,

(viii) production of immuno-modulatory cytokines,

(ix) markers of inflammation,

(x) anti-apoptotic transcription factors,

(xi) markers of ageing,

(d) comparing the results obtained with a control,

wherein a modification, decrease or inhibition of stress indicators as determined in c) is indicative for a substance capable of modifying and/or reducing/inhibiting endogenous CD_{1d} function.

25. The method according to claim 24, wherein the stress situation is a chemical stress, a biological stress or a physical stress.

26. The method according to claim 25, wherein the chemical stress is exerted by exposure to oxidants or carcinogens, or wherein the biological stress is exerted by exposure to bacteria, viruses, fungi, lipids derived from surrounding cells and/or microbes, or wherein the physical stress is exerted by exposure to UV-irradiation.

27. The method according to claim 24 to 26, wherein the pro-inflammatory cytokines/ molecules are selected from the group consisting of PAF, IL-1, TNF-α, PGE-2, IL-6, IFN-γ or IL-8.

28. The method according to any of the claims 24 to 26, wherein the immuno-modulatory cytokines are selected from the group consisting of IL-10, IL-4 or TGF-ß.

29. The method according to any of the claims 24 to 26, wherein the lipids are selected from the group consisting of phospholipids, sphingolipids and glycosphingolipids.

30. The method according to any of the claims 24 to 26, wherein the markers of inflammation include Cox-2 and iNos.

31. The method according to any of the claims 24 to 26, wherein the anti-apoptotic transcription factors include AP-1 and NFkappaB.

32. The method according to any of the claims 24 to 26, wherein the markers of aging include elastases, collagenases, metalloproteinases, gelatinases, stromelysins, telomerases.

33. Use of a substance according to any of the claims 1 to 14 or a composition according to any of the claims 15 to 17 for decreasing multi-drug resistance of cancers.

34. The use according to claim 33, wherein the cancer is skin, gut or breast cancer.

35. Use of cells expressing and/or over-expressing CD_{1d} in an assay for screening for substances modifying and/or reducing/inhibting CD_{1d} function.

36. Use of $CD_{1d}^{-/-}$ animals as a test model for determining the activity of substances influencing damages in epithelial tissues produced by a stress situation and/or hair loss.

37. Use of a substance according to any of the claims 1 to 14 in gene therapy.

38. The use according to claim 37, wherein the substance is a polynucleotide anti-sense to a sequence comprised by the CD_{1d} gene and/or the CD_{1d} mRNA.

39. Use of a gene reporter assay for screening substances stimulating CD_{1d} promoter activity.

EPO - Munich
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Summary

The present invention pertains to a method for preventing and/or treating epithelial tissue damage, such as is effected by inflammatory reactions, ageing or cancer and/or to prevent and/or treat hair loss. In particular, the present invention relates to substances and/or compositions modifying, in particular reducing/inhibiting/blocking endogenous CD_{1d} function. According to another aspect the present invention also provides a method for screening for compounds suitable for use in the method and the composition of the present invention.

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